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(54) Title: OSTEOPONTIN-BASED COMPOSITIONS FOR ENHANCING BONE REPAIR

(57) Abstract

The present invention is directed to bioactive composition that induces the repair of damaged or diseased connective tissues upon contact of the damaged or diseased tissues with the composition *in vivo*. More particularly the present invention is directed to the use of a composition comprising an effective amount of osteopontin to enhance the repair of damaged or diseased bone.

OSTEOPONTIN-BASED COMPOSITIONS FOR ENHANCING BONE REPAIR

Technical Field

This invention relates to implantable biocompatible compositions that induce the repair of damaged or diseased bone or cartilage or other connective tissues upon contact of the damaged or diseased tissues with the composition *in vivo*. More particularly the present invention is directed to the use of a composition comprising an effective amount of osteopontin to induce endogenous repair of damaged or diseased bone and cartilage tissues.

Background of the Invention

Currently, bone defects are typically repaired by autografts or banked bone. Autografts have a good ability to unify the bone, and physicians often prefer to use bone from sources such as the iliac crest. However, procedures using autografts suffer from several drawbacks. First, autografts require a separate harvest operation, which results in increases in operative time and the use of blood transfusions. Secondly, patients often lack adequate amounts of material for harvesting and often experience donation site morbidity. Implantation of banked bone does not require the harvest operation, but its bone healing capability is not as high as that of autografts. Therefore, it is undesirable to use banked bone in severe conditions such as nonunion.

Over the years, researchers have searched for compositions and methods for promoting bone growth without necessitating the use of autografts or banked bones. One potential source for bone growth promoting factors is the extracellular matrices of healthy bone and cartilage tissues. Extracellular bone matrix contains predominantly mineral (hydroxyapatite) and an organic matrix, where the major component of the organic matrix is collagen type I. The remaining components of bone matrices include a number of less abundant non-collagenous proteins and growth factors. For example, since the mid-1960's the osteoinductive activity of both demineralized bone matrix (DBM) and bone morphogenetic protein (BMP) has been studied. See, for example, S. Ijiri, *Influence of Sterilization on Bone Morphogenetic Protein*, Fourth World Biomaterials Congress, April 24-28, (1992). In addition to DBM and BMP, many of the bone matrix non-collagenous protein components

possess biological activity and find wide use in medical applications such as prosthetic devices, drugs, blood components, and the like.

One group of these non-collagenous proteins present in extracellular bone matrix are the sialoproteins, which comprise about 0.2% of the wet weight of bone extract. Osteopontin is an adhesive glycoprotein which binds calcium and collagen, and in particular binds very tightly to hydroxyapatite. Osteopontin is found in secreted bone matrix and has been proposed to play a role in bone repair and remodeling including the nucleation of the hydroxyapatite crystals during matrix mineralization, osteoblast attachment, and binding and activating of osteoblasts during bone remodeling. The mature protein contains a cell-binding Arg-Gly-Asp (RGD) sequence. This sequence has been termed the "cell-binding motif" because it is via this sequence that adhesion molecules bind cells to the extra-cellular matrix. Accordingly it is anticipated that osteopontin will participate in the attachment of bone cells to the matrix.

Osteopontin is structurally similar to bone sialoprotein and is enriched at the mineralization front of newly formed bone in the metaphysis. The protein can be isolated from warm blooded vertebrate bone and cartilage tissues using standard extraction techniques known to those skilled in the art. The expression of osteopontin is correlated with mineralization in bone and cartilage and its expression is stimulated by growth factors such as TGF β. Immunocytochemistry and *in situ* hybridization studies have shown that osteopontin also is enriched at bone surfaces facing osteoclasts and that osteopontin mRNA predominates in osteoblasts close to the metaphyseal/diaphyseal border where osteoblastic bone resorption is particularly pronounced. Osteopontin apparently also is produced by chondrocytes in calcified cartilage, by bone marrow cells, and by osteoblasts in fetal rat bone. Accordingly, it has been suggested that osteopontin has a role in biological mineralization.

Numerous compounds have been isolated from biological sources and have been identified has having potential bone growth enhancing activities based on the response of cultured cells to those compounds. In particular due to the association of osteopontin with developing bone in animal tissues, and the response of various cultured cells to osteopontin *in vitro*, it has been suggested that this molecule may play

a role in bone and/or cartilage development. However, predictions of *in vivo* activity that are based on *in vitro* cell culture activities have proven unreliable.

For example over the last two decades prostaglandins had been reported as both increasing bone resorption as well as increasing bone formation. Analysis of the literature references reporting the conflicting activities of prostaglandins reveals that almost all reports of bone resorption were performed *in vitro* and almost all the studies reporting bone formation were done *in vivo*. See Mark and Miller, Journal of Endocrinology Vol. 1 (1993). The studies of bone growth *in vitro* were performed with tissue/organ cultures of bone or relatively pure isolated bone cell populations. The apparent conflicting reports of the predominant skeletal affects of the prostaglandins can be explained on the basis of the limitations of the cell culture systems used to study those effects. Similarly, initial reports of TGF-β activity based on cell culture assays failed to correlate with observed *in vivo* activities. Therefore, skilled artisans appreciate that predictions of *in vivo* activity that are based on *in vitro* assays are unreliable.

Many compounds that have been isolated from connective tissues have been reported as having osteogenic properties based on the response of cells to these compounds *in vitro*. However due to the unreliable correlation between the *in vitro* and *in vivo* activity of bioactive agents, *in vitro*-established activities fail to provide sufficient guidance for selecting compounds that will exhibit the desired *in vivo* bone and cartilage repair enhancing activity. Accordingly, applicants have utilized an *in vivo* assay technique to identify bioactive agents that induce the repair of bone and cartilage tissues. Advantageously, the present *in vivo* assay avoids the use of delivery carriers, such as collagen, which are known to exhibit osteogenic properties themselves. Applicants have utilized this unique *in vivo* assay to identify compounds that enhance bone repair. More particularly, through the use of the *in vivo* assay described in Example 2, applicants have determined that osteopontin enhances the repair of bone *in vivo*.

Summary of the Invention

In accordance with the present invention, a composition comprising osteopontin is used to enhance the ability of endogenous cells to repair bone and

cartilage tissues in a warm-blooded vertebrate species. The disclosed composition is administered to a warm-blooded species, either by implanting or injecting the composition, for *in vivo* contact with the site in need of repair.

Detailed Description of the Preferred Embodiments

The present invention is directed to compositions comprising osteopontin in a substantially pure form, and the use of such compositions to enhance the repair of bone and cartilage defects *in vivo*. As used herein the term osteopontin is intended to include native osteopontin protein isolated from human or other warm-blooded vertebrates, naturally occurring isoforms of osteopontin, recombinant protein produced from osteopontin encoding nucleic acid sequences, and protein fragments/peptides of osteopontin proteins. An osteopontin gene is defined herein to include any nucleic acid sequence encoding for osteopontin, including the native gene sequences isolated from human or other warm-blooded vertebrates, any nucleic acid sequences encoding active fragments of bone sialoprotein protein, or any recombinant derivative thereof.

The compositions of the present invention can be utilized in a method for inducing the repair of damaged or defective tissues of a warm-blooded vertebrate. More particularly, osteopontin can be used to repair the tissues of orthopedic and non-orthopedic wound sites, including bone, cartilage, tendon, ligament, muscle, skin and other soft tissues. In one embodiment the compositions of the present invention are used to effectively repair fractures and fill or bridge bone defects including for example, craniofacial defects or periodontal defects, joint fractures, chondral defects, superficial chondral defects, full thickness defects, osteochondritis dissecans, minuscule tears, ligament tears, tendon tears, muscle lesions, myotendinitis junction lesions, skeletal reconstruction following secondary bone loss to infection or neoplasm, and the treatment of various bone or cartilaginous diseases such as osteoporosis.

Osteopontin may enhance the repair of these tissues either directly or indirectly. For example, osteopontin may increase new bone formation at a localized site by directly stimulating osteoblast activity (i.e., by enhancing matrix production or by recruiting additional osteoblast cells), by increasing angiogenesis, or by inhibiting osteoclast resorption. In addition, the compositions of the present invention may

participate in the recruitment of bone progenitor cells or bioactive agents to the localized site either by selective binding of osteopontin to the progenitor cells or the bioactive agent, or osteopontin may participate in the recruitment of cells through chemotaxis. It is also anticipated that osteopontin can be used in a wound repair context in combination with a carrier material such as a ceramic or polymer, including the use of proteins such as collagen as the carrier material. In addition osteopontin can be combined with autologous cells (such as bone or cartilage progenitor cells) or autologous proteins (such as fibrin).

In one embodiment the compositions of the present invention comprise a delivery vehicle and a bioactive mixture comprising an effective amount of a substantially pure osteopontin. In an alternative embodiment, the compositions of the present invention comprise a delivery vehicle and a bioactive mixture comprising an osteopontin gene. Delivery vehicles suitable for use in delivering bioactive agents to bone and cartilage *in vivo* are well known to those skilled in the art. In one embodiment the delivery vehicle comprises a polymer matrix, and the polymer matrix is formed from one or more biocompatible polymers. As used herein, biocompatible means that the polymer is non-toxic, non-mutagenic and elicits a minimal to moderate inflammatory reaction. Preferably the biocompatible polymer is also biodegradable and completely degrades in a controlled manner into non-toxic residues. In this embodiment, the polymer matrix serves as a delivery vehicle for the bioactive mixture, concentrating the bioactive agent at a localized site of administration and controlling the release of the bioactive composition. The controlled delivery and release of osteopontin to localized bone and cartilage sites is based on the use of biodegradable, biocompatible polymers in combination with bioactive molecules to achieve both efficacious release of molecules and removal of the polymer from the treatment site within a physiologically useful time period.

A variety of polymers can be used to form the implant for the purposes of delivering bioactive molecules to a predetermined *in vivo* site, including polyesters, polyvinyl acetate, polyacrylates, polyorthoesters, polyhydroxyethylmethacrylate (polyhema) and polyanhydrides. One of the advantages of polyesters in such applications is that they are both biodegradable and biocompatible. Aliphatic polyesters have been widely used in the area of biomaterials for implantable drug delivery devices,

sutures, and general tissues supports, after injury or surgery. The polyesters traditionally of greatest interest for localized delivery of biomaterials, are derived from lactide, glycolide, and ϵ-caprolactone monomers, with a fairly broad range of degradation profiles accessible through various termonomer combinations. The ester linkages in these aliphatic polyesters are hydrolytically and/or enzymatically labile and render the polymers degradable in aqueous environments.

In one preferred embodiment, polymers such as polyester anhydrides or ionomers are used. Alternatively, other polymers such as polylactic acid and polyorthoesters can also be used. In one embodiment the polymer matrix comprises collagen fibers. Collagen has been reported to exhibit bioactive properties and enhances the repair of bone and cartilage tissues *in vivo*. Accordingly collagen fiber can function as both as a component of the delivery system as well as an active agent of the present bone and cartilage repair compositions. Other polymer suitable for use in forming the polymer matrix comprise fibrins, starches, alginate and hyaluronic acid.

The composition of the polymer used to form the delivery vehicle matrix, as well as the molecular weight and physical properties of the polymer, can be varied according to the application. For example, hydrophobic polyanhydrides can be used where it is desirable to increase the time of degradation. Compounds can be mixed into, or polymerized with the polymer as required for additional strength or other desirable physical properties, using materials known to those skilled in the art from studies involving bone cements. For example, tricalcium phosphate or other ceramic type materials that provide better physical handling can be added to the composition.

In general, for repair of bone breaks, the polymer should release the material over a period of approximately 3 to 42 days (generally 6 weeks are required for sufficient repair to occur in humans before the bone is capable of bearing weight). The polymer should also degrade completely over a period no longer than about sixteen to twenty weeks. Release and degradation times will depend in part upon the polymer used and the bioactive materials to be released.

In accordance with one embodiment of the present invention, the delivery vehicle comprises polyester ionomers (salts of carboxy-terminated polyesters). The polyester ionomers exhibit good solubility even at higher molecular weights

dictated by implant structural/functional requirements. The polyesters are prepared from and degrade into naturally occurring metabolites for enhanced biocompatibility. The polyester ionomers are prepared from the corresponding carboxy-terminated polyesters by neutralization or partial neutralization with biocompatible, pharmaceutically acceptable salt-forming bases. In one embodiment the delivery vehicle comprises biodegradable carboxy-terminated polyesters in combination with the corresponding ionomers. The physical properties of polyester ionomers can be controlled by the degree of neutralization of the corresponding carboxy-terminated polyesters and to some extent by selection of the neutralizing base. The polyester ionomers can be used alone or in combination with their carboxy-terminated polyester precursor for use in construction of a biocompatible delivery vehicle for tissue repair and/or prolonged release of biologically active compounds.

The use of polyester ionomers as delivery vehicles is described in U.S. Patent No. 5,668,288, the disclosure of which is incorporated herein by reference. In general the polyester ionomers, is a divalent residue of a polyester. The polyester can comprise a homopolymer, copolymer, or terpolymer of biocompatible hydroxy acids, for example, lactic acid, glycolic acid, ϵ-hydroxy caproic acid, and γ-hydroxy valeric acid. Alternatively, the polyester can be formed using copolymerization of a polyhydric alcohol and a biocompatible polycarboxylic acid. Most typically such copolymers are formed between dihydric alcohols, for example, propylene glycol for biocompatibility and biocompatible dicarboxylic acids.

The bioactive component of the present compositions comprises osteopontin, optionally combined with a pharmaceutically acceptable carrier, solubilizing agent, or filler material. To induce bone growth formation osteopontin should be administered at a concentration ranging from about 10 ng to about 1 mg/ml of the defect area. In one embodiment osteopontin is administered in a concentration ranging from about 5 ug to about 100 ug/ml of the defect area. In addition, tricalcium phosphate, hydroxyapatite, gypsum, or other suitable physiological mineral sources can be combined with the compositions to assist in repair of damaged or diseased bone. In accordance with one embodiment, a physiological compatible mineral comprises up to 80% of the bioactive mix of the present composition. Alternatively, the physiological compatible mineral may comprises about 5% to about 50% of the bioactive mix, and

more preferably comprises about 5% to 30% of the bioactive mix. In addition, the present compositions can be combined with known pharmaceuticals and bioactive agents to create a delivery system for the local treatment of bone disorders or diseases.

In addition, the bioactive component of the present compositions can be further combined with growth factors, growth factor binding proteins or eukaryotic cells. Examples of suitable growth factors comprise: fibroblast growth factor, transforming growth factor (e.g., TGF-β_1), bone morphogenetic protein, epidermal growth factor or platelet-derived growth factor. Examples of growth factor binding proteins are insulin-like growth factor binding proteins (IGFBP's) such as IGFBP's 3 and 5. Examples of suitable eukaryotic cells comprise bone marrow cells, osteoblasts and mesenchymal stem cells. The bioactive composition of the present invention can further include an osteogenic agent that stimulates or accelerates generation of bone upon implantation into a bone defect site. Examples of osteogenic agents comprise demineralized bone powder, morselized cancellous bone, aspirated bone marrow, bone or cartilage forming cells, and other bone sources.

The bioactive compositions of the present invention are utilized in one embodiment for stimulating the growth of bone and cartilage tissues at a predetermined localized site in a warm-blooded vertebrate. The method comprises contacting the site in need of repair with a composition comprising substantially pure osteopontin. In one embodiment the composition is surgically implanted at the site in need of repair and the composition comprises osteopontin and a polymer matrix, wherein the polymer matrix controls the release of osteopontin and concentrates osteopontin at the desired site. Alternatively, the composition may be in an injectable form and the method of contacting the site in need of repair comprises injecting the composition into or adjacent to the site. The injectable form of the present composition typically comprises osteopontin in combination with a pharmaceutically acceptable carrier. The viscosity of the compositions can be adjusted by controlling the water content of the compositions or by the addition of pharmaceutically acceptable fillers or thickening agents known to those skilled in the art. In one embodiment, the injectable forms include collagen fibers and the viscosity of the composition is controlled by adjusting the pH of the composition to about 6.0 to about 7.5.

The compositions of the present invention can be combined with an effective amount antibiotics, chemotherapeutic agents, additional growth factors, antigens, antibodies, enzymes or hormones. For example, a composition comprising osteopontin and an antibiotic may be useful in the treatment of osteomyelitis, thereby reducing the need for and risk of parenteral antibiotics. In addition, a composition comprising osteopontin and an antineoplastic agent could be used for the local treatment of bone neoplasm, or a composition comprising osteopontin and an osteogenic or other growth factor (e.g., osteogenin, bone morphogenetic protein, parathyroid hormone, or TGFβ) could be used to accelerate the repair of skeletal defects as occurs with excessive trauma and with skeletal deficiency disorders such as osteogenesis imperfecta and osteoporosis.

As noted above the present compositions can be prepared in fluid forms for injection into a warm-blooded vertebrate. In one embodiment the injectable forms are used to systemically treat a warm-blooded vertebrate and provide therapeutic value for conditions such as osteoporosis, arthritis or other pathogenic situations that involve bone and/or cartilage. The injectable pharmaceutical formulation may be administered via the parenteral route, including subcutaneously, intraperitoneally, intramuscularly and intravenously. Examples of parenteral dosage forms include aqueous solutions of the active agent, in an isotonic saline, 5% glucose or other well-known pharmaceutically acceptable liquid carrier. In one preferred aspect of the present embodiment, the osteopontin compound is dissolved in a saline solution containing 5% of dimethyl sulfoxide and 10% Cremphor EL (Sigma Chemical Company). Additional solubilizing agents are well-known to those familiar with the art and can be utilized as pharmaceutical excipients for delivery of the osteopontin compounds. Other delivery vehicles are contemplated for use in accordance with the present invention and can be used to administer the fluid forms of the present invention systemically to a warm-blooded vertebrate. For example the delivery vehicle may be an oral dosage form, an epidermal patch or other delivery vehicle known to those skilled in the art.

Example 1

***In Vivo* Testing of Osteopontin Osteogenic Potential**

Rat Calvarial Defect Model

To determine the osteogenic ability of osteopontin, applicants used a well established model for measuring the *in vivo* induction of endogenous growth of bone tissue. In general the model involves the formation of circular defects (approximately 6-8mm in diameter) in the parietal bones of adult (greater than 6 months in age) Sprague Dawley rats. The defect is of a critical size such that the intraosseous wound would not heal by bone formation during the life of the animal.

The surgery is conducted with sterile technique, cap, mask, gown and gloves. Animals are sedated with a cocktail of Ketoset 10ml, with 0.15ml of 100mg/ml Xylazine and 0.3ml of 10mg/ml acepromarine added, and the dosage is 0.1ml/100g body weight. If additional sedation is needed Ketoset alone is used in 0.05 ml increments. After the rats are sedated, their heads are shaved from behind the ears to the tip of the nose and laterally, ventral to the ears. A three part scrub, alternating betadine and alcohol is performed. An ointment is placed in the eyes prior to scrubbing. After the animal surface has been scrubbed, the animals are placed on V-trays with their heads positioned on a small stack of 4 x 4 gauze to make a level surgery site. The animals are immobilized by taping them to the tray using strips of tape running across the nose, ears and back.

The tray with the immobilized animal is placed under a sterile drape on the surgery table. A skin incision is made in the midline of the skull, the periosteum is scraped off and retracted to expose the midline site. An 6 or 8mm trephine is used in a micro-drill under 40 pounds or less of pressure. Irrigation of the site while drilling is necessary to avoid thermal necrosis. As the bone is cut care is taken to avoid damage to the dura and sagittal sinus. The dura should be left intact if possible. If bleeding occurs the area is packed with gelfoam for a few minutes, then removed when bleeding stops. The defect edges are then scraped smooth.

A 6-8mm circle of gelfilm is placed between the brain and the composition comprising osteopontin. Once the composition is in placed in the defect, the periosteal layer is sutured closed over the defect region using a 5-0 proline continuous suture pattern. The skin is then closed with staples. Animals are recovered

in an incubator to avoid hypothermia, and once the animals are walking, they are returned to their cages.

Method of *In Vivo* Testing of Bioactive Compositions

The compositions of the present invention were administered directly to the localized *in vivo* defect site (the calvaria defect site in the rat calvaria defect model) of adult rats through the use of ALZET osmotic pumps. ALZET osmotic pumps (ALZA Scientific Products Palo Alto, California) were implanted subcutaneously into Sprague Dawley rats on their backs, slightly posterior to the scapulae. The pumps were connected to a catheter wherein the catheter directs delivery of the pump's contents (osteopontin) into the calvaria defect to provide a local dose of about 17 ug/ml of total defect volume.

The osmotic pumps were assembled prior to implantation. The pump assembly was first filled with the osteopontin composition by attaching a syringe containing the solution to be delivered to the catheter tubing and filling the osmotic pump with the solution to be delivered. The filled osmotic pump is fitted onto its flow moderator. The pump assembly is then incubated in sterile saline (0.9%) at 37°C for at least 4-6 hours. Optimal results are obtained by priming overnight. This step ensures that the osmotic pump is pumping continuously prior to implantation and minimizes the chance of clotting within the cannula or occlusion by tissue during delivery of the test agent. The assembly is then implanted into the host animal.

The rat was anesthetized (e.g., with an intraperitoneal injection of a solution of sodium pentobarbital, 40-50 mg/kg) and the pump apparatus was implanted into a subcutaneous pocket in the midscapular area of the back of the rat. To prepare the implantation site, the skin over the implantation site was shaved and washed, and a mid-scapular incision is made into the back of the animal. A hemostat was inserted into the incision and, by opening the jaws of the hemostat, the subcutaneous tissue was spread to create a pocket for the pump. The pocket should be large enough to allow some free movement of the pump (e.g., 1 cm longer than the pump). A filled pump was inserted into the pocket and connected to a catheter. The distal end of the catheter is placed into the calvaria defect for direct delivery of the osteopontin

composition to the defect. The pump insertion site is then closed with wound clips or sutures.

The manufacturer's guidelines are followed regarding the maximum drug delivery rates and durations utilized to minimize any nutrition-impairing stress or disruption of normal behavior. After its pumping lifetime has ended, the ALZET osmotic pump is removed.

Results

Experiments were conducted using an ALZET osmotic pump model 2002 which delivered its contents (200 ul volume) over a 14-day period to the defect site. The experiment was conducted over a total of 28 days after implantation of the pump. The rats were sacrificed at day 28 and a section through the center of the defect (extending from head to tail) was viewed histologically for bone growth. Two control animal groups were used where the defect region received either saline only, or nothing at all (i.e., the pump was "empty"). The sections were scored in a blinded manner for bone growth using a scale of 0-5 wherein the score is based on the amount of new bone growth observed in accordance with the following scale:

0 = no growth or resorption of existing bone;

1 = greater than zero to about 10% of the gap bridged with bone;

2 = about 10% to about 33% of the gap bridged with bone;

3 = about 33% to about 66% of the gap bridged with bone;

4 = about 33% or greater of the gap bridged with bone;

5 = complete bridging of the gap.

Table 1 describes the experimental procedures used to analyze the *in vivo* bone growth response of rats to osteopontin. The following abbreviations are used: BW, beginning weight; SAC date, sacrifice date; DOB, date of birth; TX, treatment.

Table 1

Surgery Date	Rat #	BW (G)	SAC Date	DOB	TX	Date Received/ Recovery Time Surgery Comment	HEM	DUR
8/29/97	1	410	9/26/97	2/1/97	3	8/25/1997/2hrs/		
8/29/97	2	395	9/26/97	2/1/97	3	8/25/1997/2.5 hrs/		
8/29/97	3	375	9/26/97	2/1/97	3	8/25/1997/3.5 hrs/		
8/29/97	7	355	9/26/97	2/1/97	1	8/25/1997/3+hrs/ SQ fluids/		
8/29/97	8	385	9/26/97	2/1/97	1	8/25/1997/3+hrs/ SQ fluids/	X	X
8/29/97	9	360	9/26/97	2/1/97	1	8/25/1997/3+hrs/ SQ fluids/	X	X
9/2/97	10	355	9/30/97	1/1/97	5	7/31/1997/2.5hrs/		
9/2/97	11	400	9/30/97	1/1/97	5	7/31/1997/2.5hrs/	X	
9/2/97	12	375	9/30/97	1/1/97	4	7/31/1997/3hrs/		
9/2/97	13	375	9/30/97	2/1/97	4	8/25/1997/3hrs/		
9/2/97	14	360	9/30/97	2/1/97	4	8/25/1997/3hrs/		
9/2/97	15	330	9/30/97	2/1/97	5	8/25/1997/3hrs/		
9/3/97	19	370	10/1/97	2/1/97	1	8/25/1997/3hrs/		
9/3/97	20	370	10/1/97	2/1/97	1	8/25/1997/3hrs/		
9/3/97	21	415	10/1/97	2/1/97	1	8/25/1997/3hrs/		
9/3/97	22	385	10/1/97	2/1/97	3	8/25/1997/3hrs		
9/3/97	23	385	10/1/97	2/1/97	3	8/25/1997/3hrs/		
9/3/97	24	355	10/1/97	2/1/97	3	8/25/1997/3hrs/		
9/16/97	25	380	10/14/97	2/1/97	5	8/25/1997/4hrs/ slow rec. SQ fluids	X	
9/16/97	26	440	10/14/97	2/1/97	5	8/25/1997/3hrs/		
9/16/97	30	400	10/14/97	2/1/97	5	8/25/1997/3hrs/		
9/16/97	31	405	10/14/97	2/1/97	4	8/25/1997/3hrs		
9/17/97	35	420	10/15/97	2/1/97	4	8/25/1997/2hrs/		
9/17/97	36	415	10/15/97	2/1/97	4	8/25/1997/2hrs/		
9/17/97	37	380	10/15/97	2/1/97	2	8/25/1997/3hrs/	X	

Table 1 continued

Surgery Date	Rat #	BW (G)	SAC Date	DOB	TX	Date Received/ Recovery Time Surgery Comment	HEM	DUR
9/17/97	38	355	10/15/97	2/1/97	2	8/25/1997/3hrs/	X	
9/17/97	39	400	10/15/97	2/1/97	2	8/25/1997/3hrs/		
9/17/97	40	395	10/15/97	2/1/97	2	8/25/1997/3+hrs/		
9/17/97	41	350	10/15/97	2/1/97	2	8/25/1997/3+hrs/		
9/17/97	42	390	10/15/97	2/1/97	2	8/25/1997/3+hrs/		

Treatment	# of Animals	Description (700,000 cells = 1X)
1	6	OPN (6ug/ml) + Collastat
2	6	OPN (6ug/ml) + Collastat + 1X Percoll cells
3	6	OPN (60ug/ml) + Collastat
4	6	Autograft
5	6	Empty

The following histology scores were obtained for the experiment described in Table 1:

Osteopontin (17 ug/ml of defect):

Mean: 2.17, Standard Deviation 0.75, n=6.

Control:

Mean 1.33, Standard Deviation 0.52, n=6.

In summary the introduction of osteopontin into a rat calvarial defect via the osmotic pump method enhances new bone formation.

Example 2

Intravenous Infusion Via the External Jugular Vein

The osteopontin compositions of the present invention can also be administered intravenously to provide systemic administration of the composition. Such systemic administration may provide therapeutic value for orthopedic conditions

such as osteoporosis or other pathogenic conditions involving bone or cartilage. As described in Example 1 the ALZET pumps can deliver fluid compositions directly into the venous or arterial circulation via a catheter. ALZET pumps have been shown to pump successfully against arterial pressure with no reduction in flow. The following procedure details placement of a catheter in the external jugular vein. In many cases this site is preferable because of its size and ease of access, however, other sites may be used successfully.

An osmotic pump flow moderator is connected to one end of a catheter (inside diameter ≤ 0.07 cm). The catheter should be 25% longer than the distance between the site of subcutaneous pump implantation (the midscapular region) and the site where the catheter enters the external jugular vein. The flow moderator and catheter is filled by attaching a syringe filled with osteopontin composition to the free end of the catheter. The osmotic pump is filled with the osteopontin composition and fitted onto the flow moderator. The syringe which was used to fill the catheter can now be detached and the flow moderator inserted until the white flange is flush with the surface of the pump. The pump and catheter should be completely filled and free of air bubbles. The filled pump and catheter are incubated in sterile saline (0.9%) at 37°C for at least 4-6 hours. This step ensures that the osmotic pump is pumping continuously prior to implantation, minimizing the possibility of clotting and catheter occlusion during delivery of the test agent.

The complete assembly is then implanted into the animal as follows. The ventral portion of the animal's neck is shaved and cleaned and the neck is incised to one side of the midline, and the tissues spread along the head to tail axis. Using blunt dissection, the external jugular vein is located just beneath the skin and is elevated and cleaned for a distance of 1.5 cm. A silk ligature (3.0) is then placed around the head end of the cleaned vein and tied, and all large branches of the vein are tied off, but not cut. Two loose, overhand knots are placed at the heart end of the vein. Using the belly of sharp, curved iris scissors, the mid-portion of the vein is grasped, elevated and cut, so that an ellipsoidal piece of the vein wall is removed. (This technique is better than making a nick with the tip of the scissors.) The free end of the catheter is inserted into the hole in the vein wall, and advanced gently to the level of the heart (about 2 cm in an adult rat). The proximal (heart-end) ligatures are

tied snugly around the catheter, being careful not to crimp the catheter. The distal (head-end) ligature is then tied around the catheter. The ends of all three ligatures are then cut off close to the knots.

A hemostat is then used to tunnel over the neck creating a pocket on the back of the animal in the midscapular region. The pump is positioned into this pocket, allowing the catheter to reach over the neck to the external jugular vein with sufficient slack to permit free head and neck movement. The incision in the skin of the neck is then closed with 2 or 3 wound clips or with sutures.

CLAIMS:

1. The use of isolated osteopontin in the preparation of a composition useful for inducing endogenous growth of bone at an *in vivo* site in need of said growth.

2. The use of isolated osteopontin in accordance with claim 1 wherein the osteopontin is combined with a physiologically acceptable carrier to form an injectable composition.

3. A method for inducing localized bone growth at a predetermined *in vivo* site of a vertebrate species, said method comprising

contacting said site with a composition comprising substantially purified osteopontin, in an amount effective to induce endogenous tissue growth, and a pharmaceutically acceptable carrier.

4. The method of claim 3 wherein the composition is in fluid form and the site is contacted by injection of the composition.

5. The method of claim 3 wherein the composition further comprises a biocompatible polymer matrix.

6. The method of claim 5 wherein the polymer matrix comprises a polymer selected from the group consisting of polyesters, ionomers, poly(amino acids), polyvinyl acetate, polyacrylates, polyorthoesters, polyanhydrides, collagens, fibrins, starches, alginate and hyaluronic acid.

7. The method of claim 3 wherein the pharmaceutically acceptable carrier comprises a mineral salt, or metal or glass compound.

8. The method of claim 7 wherein the mineral salt is selected from the group consisting essentially of tricalcium phosphate, hydroxyapatite and gypsum.

9. The method of claim 5 wherein the polymer matrix comprises a biodegradable polymer.

10. The method of claim 9 wherein the biodegradable polymer is selected from the group consisting of collagens and polyester ionomers.

11. The method of claim 5 wherein the composition is surgically implanted at the site.

12. A composition for enhancing the growth of bone tissues said composition comprising a delivery vehicle and a bioactive mixture comprising an effective amount of substantially pure osteopontin.

13. The composition of claim 12 wherein the delivery vehicle comprises a polymer matrix formed from a biocompatible polymer.

14. The composition of claim 12 wherein the polymer matrix comprises a polymer selected from the group consisting of polyesters, ionomers, poly(amino acids), polyvinyl acetate, polyacrylates, polyorthoesters, polyanhydrides, collagens, fibrins, starches, alginate and hyaluronic acid.

15. The composition of claim 12 further comprising a mineral salt selected from the group consisting essentially of tricalcium phosphate, hydroxyapatite, and gypsum.

16. The composition of claim 12 wherein the polymer matrix comprises a biodegradable polymer.

17. The composition of claim 16 wherein the biodegradable polymer is selected from the group consisting of collagens and polyester ionomers.

18. The composition of claim 12 further comprising additional growth factors, growth factor binding proteins or eukaryotic cells.

19. A method for treating a bone pathogenic condition in a warm-blooded vertebrate, said method comprising

administering a composition systemically to said warm-blooded vertebrate, wherein the composition comprises substantially purified osteopontin, in an amount effective to induce endogenous tissue growth, and a pharmaceutically acceptable carrier.

20. The method of claim 19 wherein the composition is administered by parenteral injection.

21. The method of claim 19 wherein the pathogenic condition is osteoporosis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/27808

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61F 2/28
US CL :623/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 623/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
Search Terms: osteopontin and (injection or injected or inject)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,533,836 A (MOORE) 09 July 1996, entire document.	1-21
X,P	US 5,824,651 A (NANCI et al) 20 October 1998, entire document.	1, 3, 12, 13
X	US 5,372,503 A (ELIA) 13 December 1994, entire document.	1-3, 5-18
A	US 5,158,934 A (AMMANN et al) 27 October 1992, entire document.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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